In vitro Analysis of the Thyroid Hormone Receptor in Mitochondrial Transcription

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Introduction

The central dogma theory relates how DNA is transcribed into messenger RNA (mRNAs) and then translated into proteins. Since the nucleus contains the majority of the DNA in cells, research related to transcription and translation focuses on these processes within the nucleus and cytosol; however, these processes are also taking place within the mitochondrial organelle. Mitochondria are most widely known for their essential role in producing energy for the cell, but the organelle also contains its own small, circular genome. Transcription of mitochondrial DNA (mtDNA) follows similar mechanisms as does transcription of nuclear DNA. During this essential process, specific mitochondrial transcription factors, such as mtTFA and mtTFB2, regulate the attachment of the mitochondrial RNA polymerase (POLRMT) to the promoter and initiation of transcription (Shutt, *et al.*, 2010). With a fully functioning mitochondrial RNA polymerase, transcription is properly conducted, and transcripts can be translated to protein by the mitochondrial ribosome (Bestwick & Shadel, 2013). Mitochondrial transcription is a major regulatory process within the organelle, and determining transcription factors involved in this control point is important for understanding mitochondrial function and many diseases relating to mitochondrial dysfunction.

Numerous transcription factors are found both in the nucleus as well as in the mitochondrial where their function is not well understood. One such transcription factor is the thyroid hormone receptor (Psarra & Sekeris, 2008). Previous research suggests that when the hormone triiodothyronine (T3) is present and taken up in cells, mitochondrial transcription increases (Enriquez, *et al.*, 1999). The mechanism behind the T3 stimulation of transcription is thought to be a coordinated effect by interacting with both the mitochondrial and nuclear thyroid hormone receptor. *Our aim is to analyze the level of interaction that the mitochondrial thyroid hormone receptor (mt-TRalpha1) has with the mitochondrial DNA and other core mitochondrial transcription factors in the presence and absence of the T3 hormone.* With this information, we would further understand another component of mitochondrial transcription that could have implication in mitochondrial dysfunction and disease.

Methods and Materials

Generating the mtDNA template plasmids:
Total DNA was extracted from HepG2 cells and PCR was completed using primers to produce the mtDNA templates shown in Figure 1C. Both the linear templates and the pGEMT plasmid (Promega) were restriction digested using EcoRI enzyme individually. DNA ligation followed by transformation to *E. coli* cells was done to obtain a single plasmid with mitochondrial DNA insert.

In vitro transcription using non-radioactive materials:
The pGEM Express Positive Control transcription template was generated and used in the standard protocol with either T7 or SP6 RNA polymerase (Promega). In vitro transcription reactions were directly run on formamide denaturing gels and stained with Gel Red (Biotinium).

Expression and purification of mt-TRalpha1:
JM109 *E. coli* cells harboring the mt-TRalpha1 gene on a plasmid were induced for protein over-expression by the addition IPTG to the growth media. Cells were collected and the purification of mt-TRalpha1 protein was attempted through subsequent denaturing steps using guanidine-HCl followed dialysis to renature the protein (Daadi, *et al.*, 1995).

Results

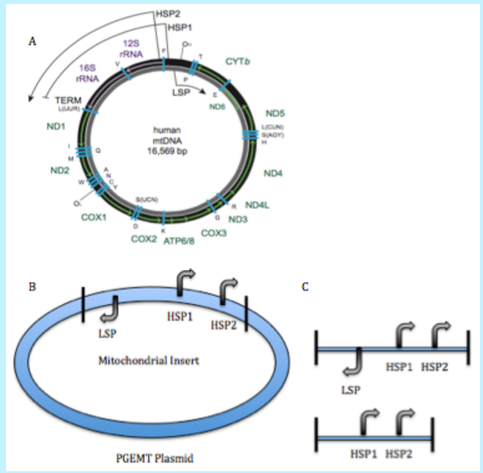

Figure 1: (A) Details the complexity of Human Mitochondrial DNA indicating specific promoter directionality for transcription. (B) Outlines a simple rendering of a template containing mtDNAligated in a pGEMT plasmid. (C) Displays the unique mtDNA fragments containing either HSP1/HSP2/LSP or only HSP1/HSP2 promoters, which will be inserted into the PGEMT plasmid. ECORI restriction enzyme sites are represented as solid black lines in parts (B) and (C).

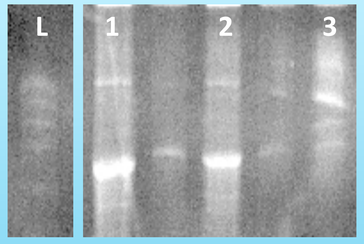

Figure 2: in vitro transcription using the pGEM Express Positive Control template and protocol (Promega) with the T7 polymerase in lanes 1 and 2, and the SP6 polyermase in lane 3. (L shows transcript RNA markers, Simga Aldrich).

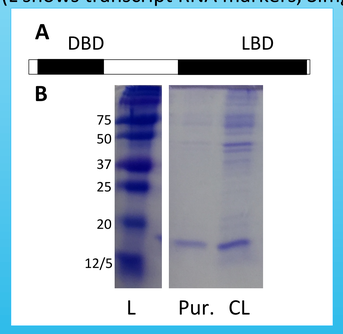

Figure 3: (A) Representation of the thyroid hormone receptor (mt-TRalpha1). DBD, DNA binding domain; LBD, ligand binding domain for the T3 thyroid hormone. (B) Coomassie stained SDS-PAGE gel of cell lysate from JM109 *E. coli* cells expressing mt-TRalpha1. L, molecular weight markers (kDa); Pur., purfied sample; CL, cell lysate

Conclusions & Future Directions

- Upon final generation of the mitochondrial DNA templates, non-radioactive methods developed using the T7 and SP6 RNA polymerases will be used for in vitro mitochondrial transcription. These reactions will include the core mitochondrial transcription machinery (POLRMT, mt-TFB2, mt-TFA) and purified mt-TRalpha1.
- The current method for purifying mt-TRalpha1 leads to a heterogeneous mixture of proteins. To generate a homogenous sample of recombinantly expressed and purified mt-TRalpha1, a protein-tagged version of mt-TRalpha1 will be generated using a six-His tag and affinity chromatography will be used for better purification.
- With homogenous purification of the mt-TRalpha1 protein, DNA binding assays will also be used to ensure the transcription factor is binding the the mtDNA templates generated.

References

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